Electronic Supplementary Material (ESI) for New Journal of Chemistry.

Supporting Information

For

Self-assembly of a novel Cu (II) co-ordination complex forms metallo-vesicles that are able to transfect mammalian cells

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Characterization techniques

TEM experiments were performed using a JEOL JEM3200FS Electron Microscope using uranyl acetate (1%) in water as the negative stain. 200 mesh carbon coated copper grids were used for analysis. The TEM was operated at 200 kV accelerating voltage. A drop of an aqueous 1mM dispersion of Cu-complex 1 metallovesicles was placed on carbon-coated 200 mesh copper grid, stained with 1% Uranyl acetate allowed to adsorb and the surplus was removed by filter paper and let it to air dry before imaging.

Fluorescence microscopy experiments were performed on Zeiss Fluo-Tec Fluorescent microscope. Flow cytometry analysis was performed with Cytomics FC500. Data acquisition and analysis was performed using the CXP software (Beckman Coulter, Miami, FL). Confocal laser microscope (LSM 700 and assisted with ZEN 2009 software (Zeiss, New York, NY) was used to observe the co fluorescence of DNA-metallo-vesicle. Single-crystal X-ray diffraction data for the compounds were collected on a Nonius Kappa CCD diffractometer equipped with Mo Ka radiation (λ = 0.71073 Å). Nuclear Magnetic Resonance (NMR) spectrum was obtained using Bruker 400 MHz NMR spectrometer. Deutearated chloroform was used the solvent for the NMR study. PerkinElmer FT-IR spectrum--100 spectrometer (equipped with ATR accessory) was used to obtain Fourier transform infrared (FTIR) spectra. Neat solid samples were used for FTIR analysis. UV-Vis spectrum was obtained by using Variant Cary 50 UV-Vis Spectrophotometer using quartz cuvette of path length 1 cm. Zeta potential measurement was carried out on 0.1mM metallo-vessicles dispersion in water using Zetasizer Nano ZS90 with Disposable folded capillary cell.

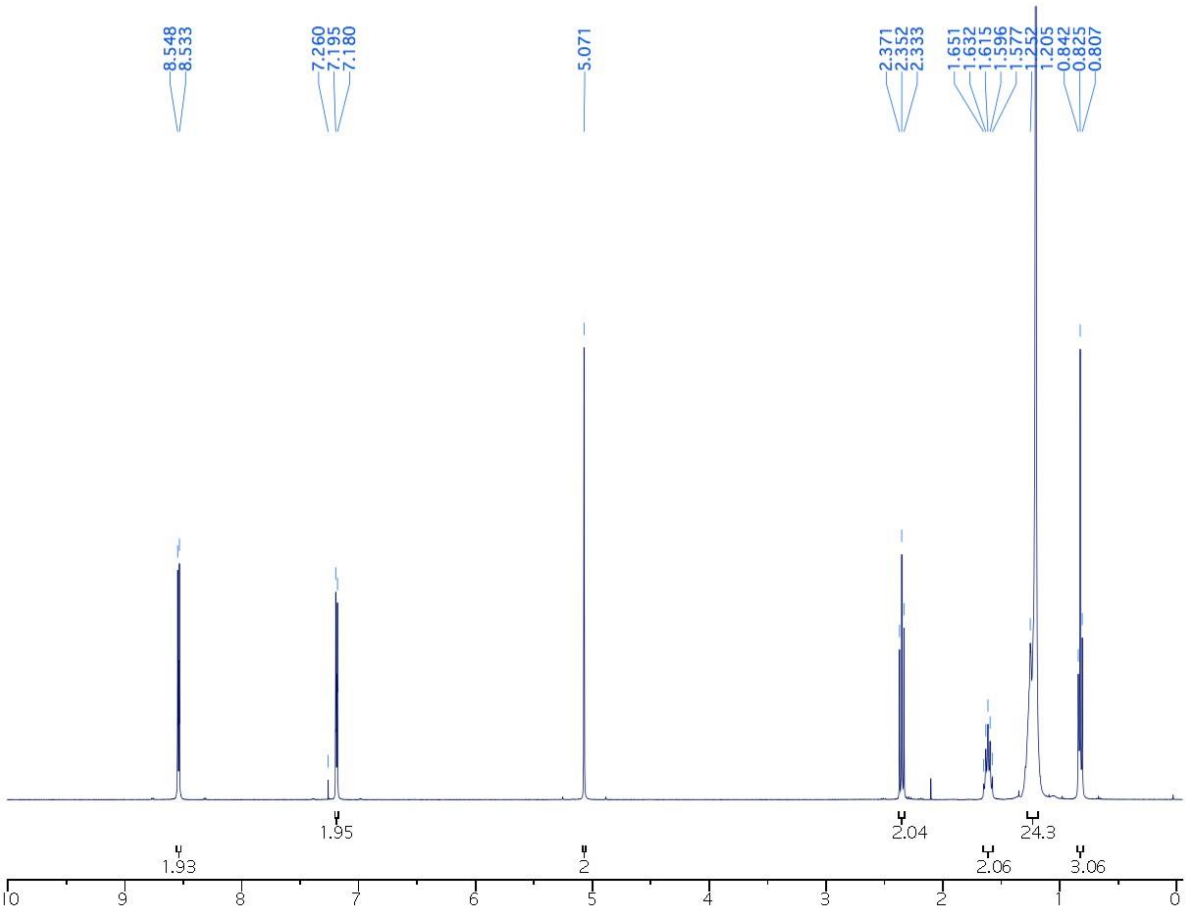

Figure S1: ^{1}H-NMR of 4-pyridylmethyl hexadecanoate in $CDCl_3$

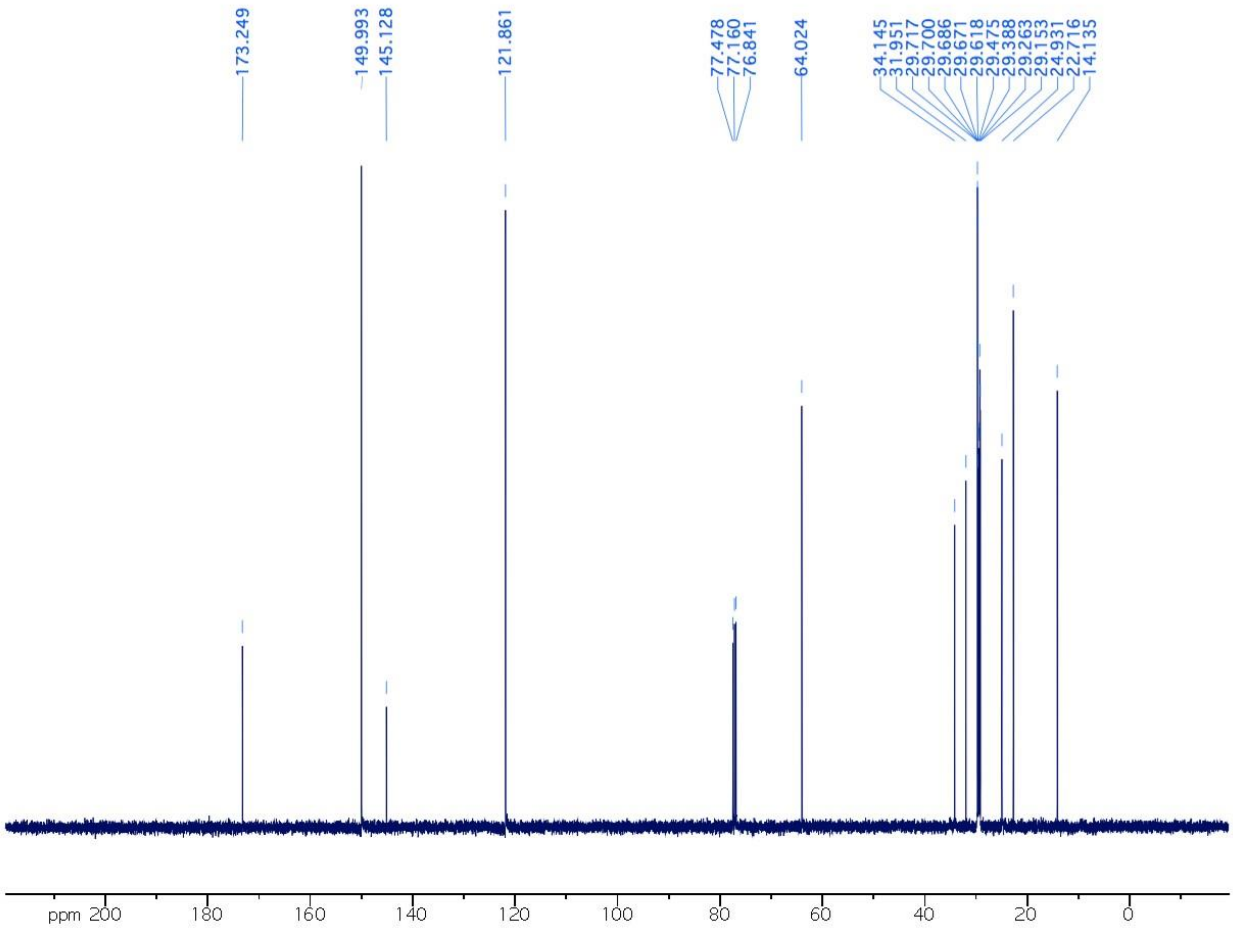

Figure S2: ^{13}C-NMR of 4-pyridylmethyl hexadecanoate in $CDCl_3$

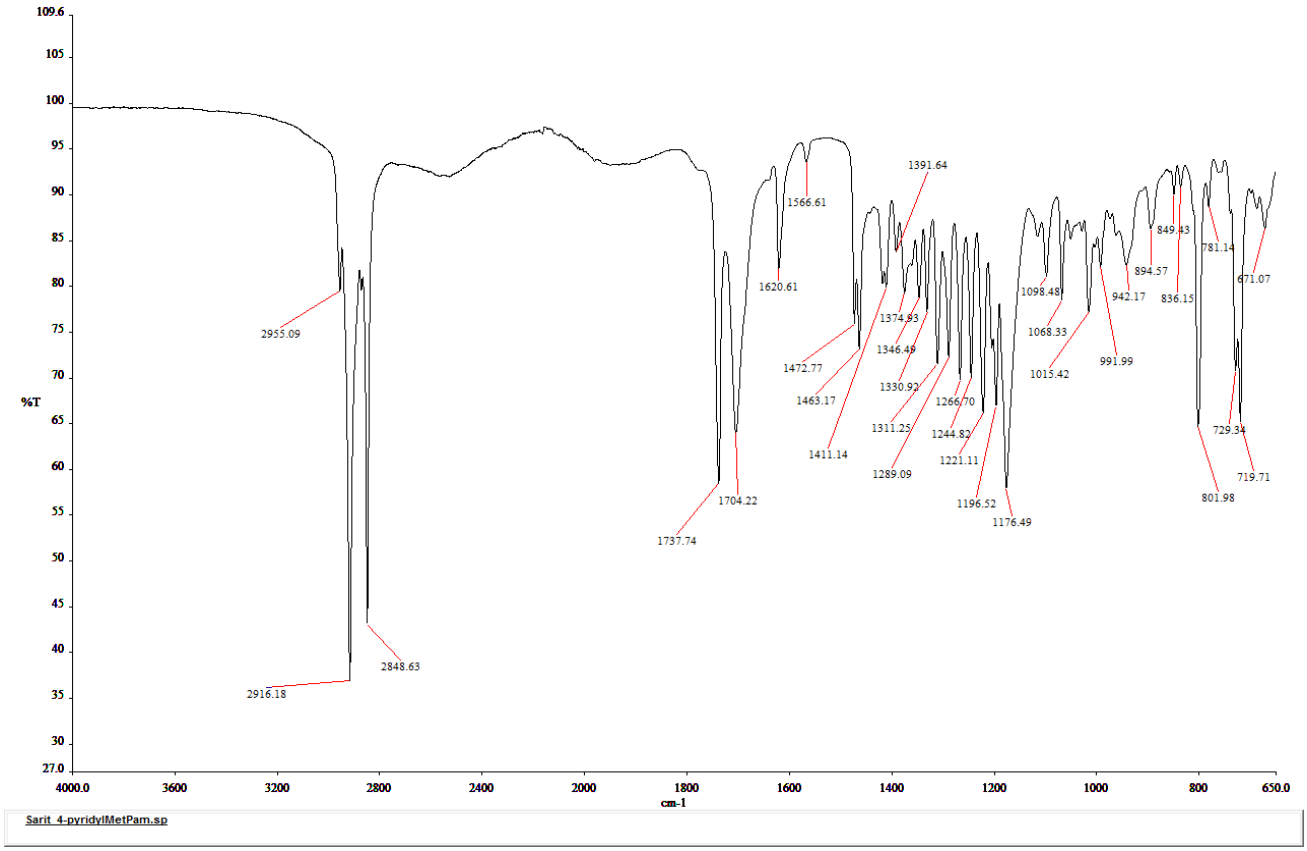

Figure S3: FTIR of 4-pyridylmethyl hexadecanoate.

FTIR (neat, cm^{-1}, supporting information, Figure S3): 2955 (w, aliphatic C-H stretch); 2916 (s, aliphatic C-H stretch), 2848 (s, aliphatic C-H stretch); 1737 (s, C=O stretch), 1620, 1566 (w, aryl C=N stretch), 1472 (w, C-H scissoring), 1463 (m, aryl C=C stretch), 1266, (m, ester C-O stretch), 1171 and 1112 (m, C-O bend), 1068 (m, ester C-O bend).

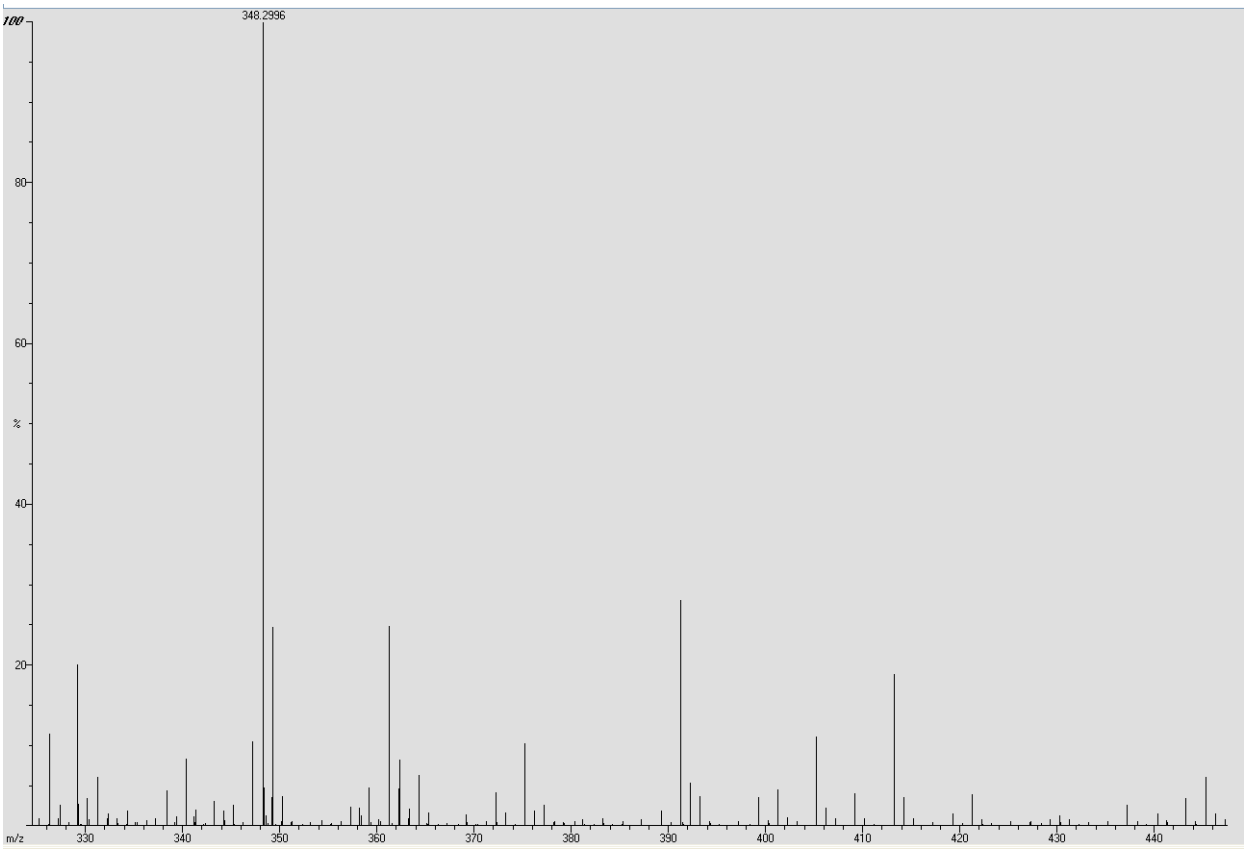

Figure S4: ESI-mass spectra of 4-pyridylmethyl hexadecanoate showing (M+1) peak at m/z= 348.2996

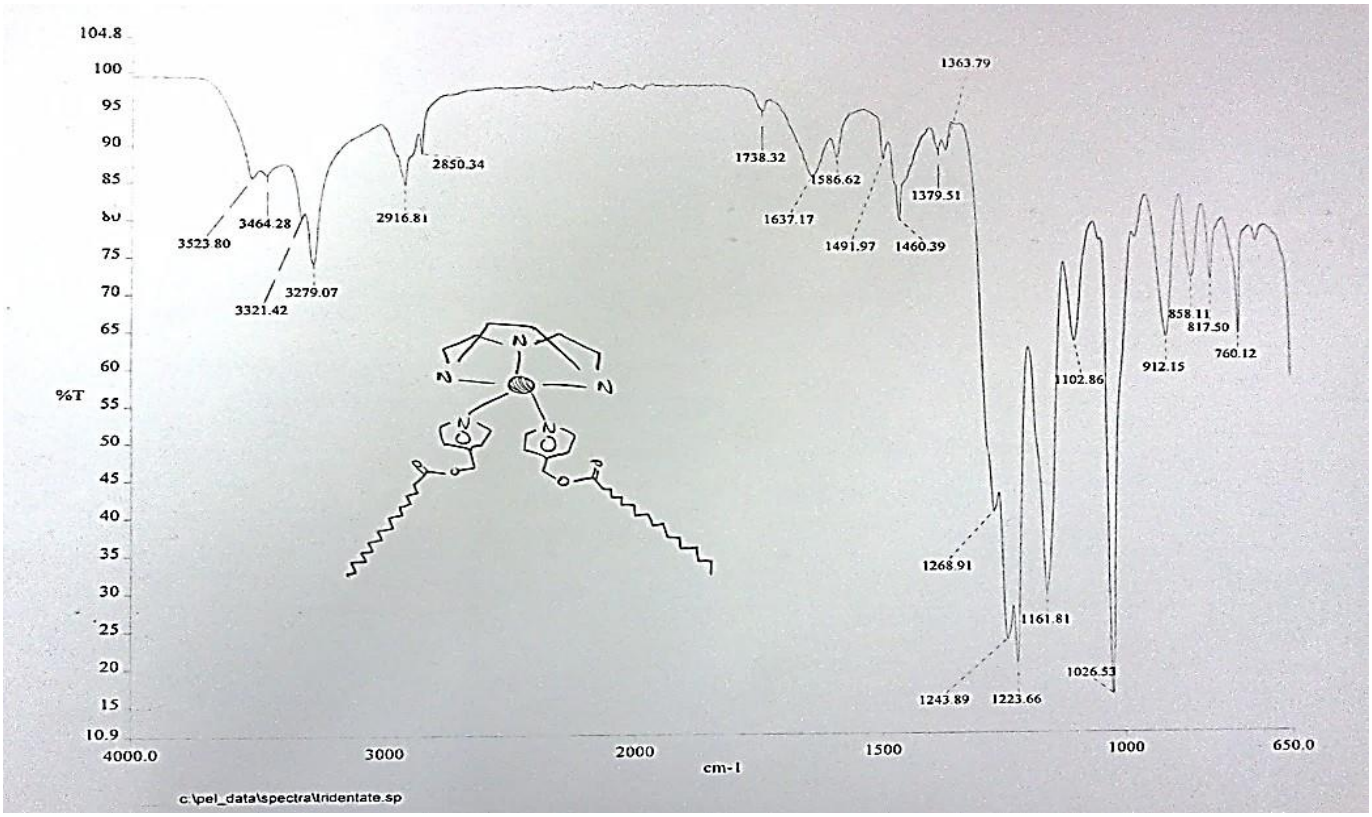

Figure S5: FTIR of bis-(4-(pyridyl)methylhexadecanoate)-(1,4,7-triazacyclononane) copper (II) triflate (complex 1).

FTIR : Additional peaks characteristic to the triazacyclononane and trifluoromethanesulfonate emerged in the FTIR spectra of the complex 1. For example, secondary amine N-H stretching from 3279 to 3523 cm^{-1}, C-N stretching at 1161 cm^{-1}, N-H wagging 760 cm^{-1}, S-O stretching of SO_3H at 1269 and 1026 cm^{-1}, C-F stretching of CF_3 at 1243 and 1161 cm^{-1}. UV-Vis in CH_3CN, λ_{max} =580 nm.

DNA mobility shift assay:

Method:

Interaction between DNA and any given molecule forming a DNA complex are investigated by observing the retardation of DNA migration using agarose gel electrophoresis [1]. The experimental reaction mixtures, having 10 µl total volume each, containing final concentration of 1mM, 50 µM of complex 1 and 50 µM extruded complex 1 metallo-vesicle suspension, each mixed with 500 ng of plasmid DNA (pEGFPN1) in PBS pH 7.4 followed by 30 min incubation at 37ºC. Then 2 µl of 6X gel loading buffer is added in each reaction mixture tubes placed on ice. The samples were analyzed by using 1 %-(w/v)-agarose-gel electrophoresis dissolved in TAE buffer (0.04 M Tris base, 0.04 m acetate and 0.001 M EDTA) pH 8.0. Ethidium bromide was present in the agarose gel electrophoresis at a concentration of 0.5 µg/ml, for staining DNA purposes. Gel electrophoresis was done at 80V for 90 minutes. DNA was visualized with gel documentation systems and a picture captured under UV-light trans-illuminator (Alpha Innotech, San Leandro, CA).

As a positive control, well known DNA intercalator compound, 4',6-diamidino-2-phenylindole (DAPI) 2.5 µl mixed with 500 ng pEGFPN1 plasmid solution was incubated and as a negative control we used only 500 ng of plasmid mixture.

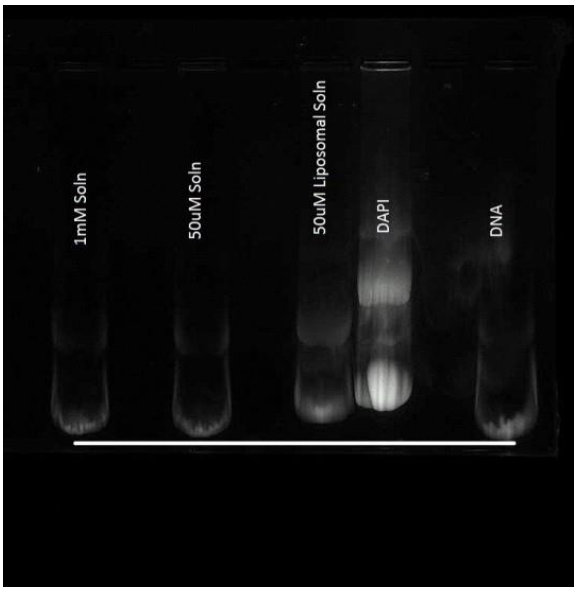

Figure S6: DNA mobility shift assay

Result and Discussion:

Migration of negative control (only DNA) is highest and same with 1 mM and 50µM compound solution incubated with DNA, it indicates that the prepared compound solutions do not bind with DNA. It is well known that migration depends upon the mass of DNA molecule and voltage applied on the agarose electrophoresis field. In this experiment voltage is a fixed parameter. So, comparing with the migration of positive control [DAPI, a DNA intercalator] with 50µM Cu-tridentate liposomal sample incubated with plasmid DNA demonstrate that in both the cases increased molecular weight of DNA due to binding with the molecules causes less migration on the electrophoresis field. Though experimental liposomal solution shows greater migration than positive control but lesser migration than negative control. This result confirms that 50µM Cu-tridentate liposomal solution has the property to bind with DNA.

Table 1. Crystal data and structure refinement for complex 2

Empirical formula	C18 H25 Cu F6 N5 O6 S2	
Formula weight	649.09	
Temperature	150(1) K	
Wavelength	0.71073 Å	
Crystal system	Monoclinic	
Space group	$P2_1/n$	
Unit cell dimensions	a = 9.04340(10) Å	a= 90°.
	b = 23.9537(5) Å	b= 102.9958(12)°.
	c = 12.3526(2) Å	g = 90°.

Volume	2607.32(7) $Å^3$
Z	4
Density (calculated)	1.654 Mg/m^3
Absorption coefficient	1.084 mm^{-1}
F(000)	1324
Crystal size	0.30 x 0.25 x 0.15 mm^3
Theta range for data collection	3.06 to 27.47°.
Index ranges	-11<=h<=11, -31<=k<=28, -16<=l<=16
Reflections collected	10534
Independent reflections	5923 [R(int) = 0.0227]
Completeness to theta = 27.47°	99.4 %
Absorption correction	Multi-scan
Max. and min. transmission	0.8542 and 0.7368
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	5923 / 0 / 444
Goodness-of-fit on F^2	1.032
Final R indices [I>2sigma(I)]	R1 = 0.0368, wR2 = 0.0853
R indices (all data)	R1 = 0.0495, wR2 = 0.0930
Extinction coefficient	0.0024(5)
Largest diff. peak and hole	0.552 and -0.708 $e.Å^{-3}$

Table 2. Selected Bond lengths [Å] and angles [°] for Cu complex **2.**

Cu(1)-N(4)	2.0301(19)
Cu(1)-N(1)	2.0328(19)
Cu(1)-N(5)	2.0371(19)
Cu(1)-N(2)	2.0462(19)
Cu(1)-N(3)	2.259(2)
N(4)-Cu(1)-N(1)	162.77(8)
N(4)-Cu(1)-N(5)	92.84(7)

N(1)-Cu(1)-N(5)	91.39(8)
N(4)-Cu(1)-N(2)	92.56(8)
N(1)-Cu(1)-N(2)	83.30(8)
N(5)-Cu(1)-N(2)	174.58(8)
N(4)-Cu(1)-N(3)	113.77(8)
N(1)-Cu(1)-N(3)	82.31(8)
N(5)-Cu(1)-N(3)	96.25(8)
N(2)-Cu(1)-N(3)	82.03(8)

Zeta potential measurements of the metallo-vesicles:

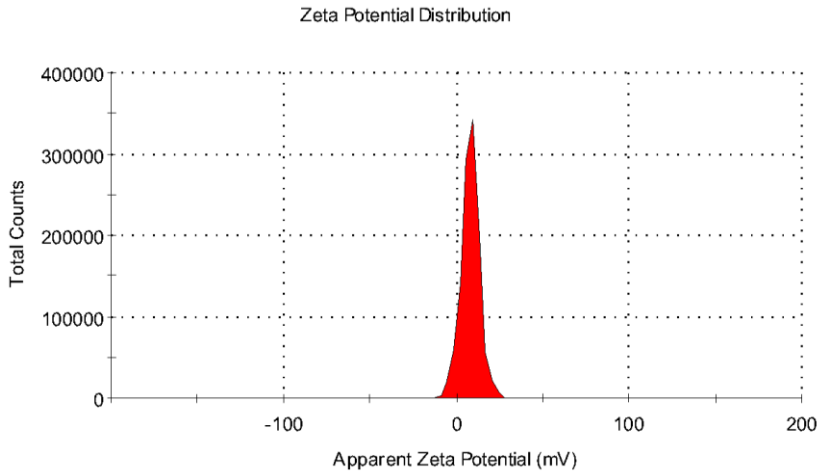

Figure S7: Zeta potential of metallo-vesicles (0.1mM) in water.

Reference:

1. Keyhani, J.; Jafari-Far, F.; Einollahi. N.; Ghadirian,E.; Keyhani, E.; DNA-mobility shift assay and the detection of anti-DNA IgG in systemic lupus erythematosus patients. Immunol Letters.1998 Jun; 62(2):81-6